Lecture No. 2

Fermentation

A bioreactor refers to any manufactured device or system that supports a biologically active environment In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel.

It may also refer to a device or system designed to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical/bioprocess engineering.

On the basis of mode of operation, a bioreactor may be classified as batch, fed batch or continuous (e.g. a continuous stirred-tank reactor model). An example of a continuous bioreactor is the chemostat.

Organisms growing in bioreactors may be submerged in liquid medium or may be attached to the surface of a solid medium. Submerged cultures may be suspended or immobilized. Suspension bioreactors can use a wider variety of organisms, since special attachment surfaces are not needed, and can operate at a much larger scale than immobilized cultures. However, in a continuously operated process the organisms will be removed from the reactor with the effluent. Immobilization is a general term describing a wide variety of methods for cell or particle attachment or entrapment. It can be applied to basically all types of biocatalysis including enzymes, cellular organelles, animal and plant cells. Immobilization is useful for continuously operated processes, since the organisms will not be removed with the reactor effluent, but is limited in scale because the microbes are only present on the surfaces of the vessel.

Design

Bioreactor design is a relatively complex engineering task, which is studied in the discipline of biochemical/bioprocess engineering. Under optimum conditions, the microorganisms or cells are able to perform their desired function with limited production of impurities. The environmental conditions inside the bioreactor, such as temperature, nutrient concentrations, pH, and dissolved gases (especially oxygen for aerobic fermentations) affect the growth and productivity of the organisms. The temperature of the fermentation medium is maintained by a cooling jacket, coils, or both. Particularly exothermic fermentations may require the use of external heat exchangers.

Nutrients may be continuously added to the fermenter, as in a fed-batch system, or may be charged into the reactor at the beginning of fermentation. The pH of the medium is measured and adjusted with small amounts of acid or base, depending upon the fermentation. For aerobic (and some anaerobic) fermentations, reactant gases (especially oxygen) must be added to the fermentation. Since oxygen is relatively insoluble in water (the basis of nearly all fermentation media), air (or purified oxygen) must be added continuously. The action of the rising bubbles helps mix the fermentation medium and also eliminates waste gases, such as carbon dioxide.

In practice, bioreactors are often pressurized; this increases the solubility of oxygen in water. In an aerobic process, optimal oxygen transfer is sometimes the rate limiting step. Oxygen is poorly soluble in water—even less in warm fermentation broths and is relatively scarce in air (20.95%). Oxygen transfer is usually helped by agitation, which is also needed to mix nutrients and to keep the fermentation homogeneous. Gas dispersing agitators are used to break up air bubbles and circulate them throughout the vessel.

Fouling can harm the overall efficiency of the bioreactor, especially the heat exchangers. To avoid it, the bioreactor must be easily cleaned. Interior surfaces are typically made of stainless steel for easy cleaning and sanitation. Typically, bioreactors are cleaned between batches, or are designed to reduce fouling as much as possible when operated continuously. Heat transfer is an important part of bioreactor design; small vessels can be cooled with a cooling jacket, but larger vessels may require coils or an external heat exchanger

Bioreactor – can be described as a vessel which has provision of cell cultivation under sterile condition & control of environmental conditions e.g., pH, Temperature, Dissolved oxygen etc.

It can be used for the cultivation of microbial plant or animal cells. This process can either be aerobic or anaerobic. The bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel.

Specifications of a bioreactor: A typical bioreactor consists of following parts:

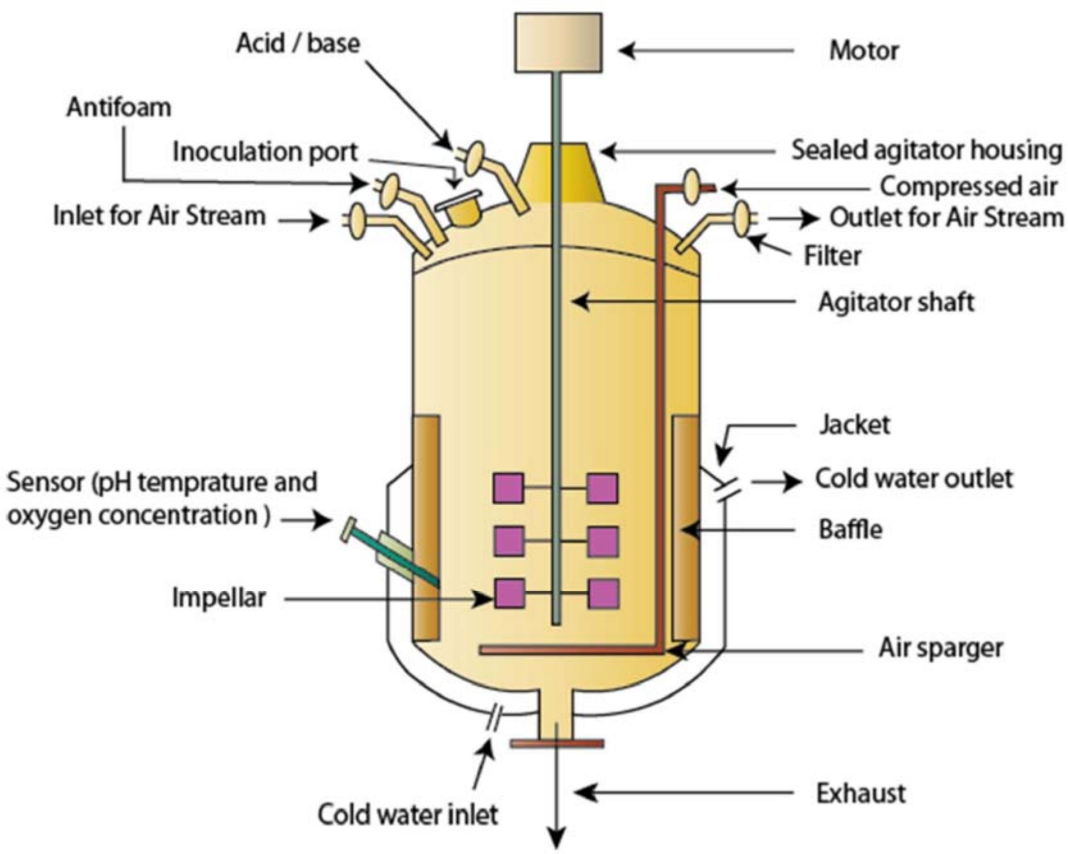
Agitator – used for the mixing of the contents of the reactor which keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen to the desired product(s).

Baffle – used to break the vortex formation in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.

Sparger – In aerobic cultivation process, the purpose of the sparger is to supply adequate oxygen to the growing cells.

Jacket – The jacket provides the annular area for circulation of constant temperature of water which keeps the temperature of the bioreactor at a constant value

Diagram of a typical bioreactor



Operational stages in a bio-process

A bioprocess is composed mainly of three stages: upstream processing, bio reaction, and downstream processing to convert raw material to finished product. After upstream processing step, the resulting feed is transferred to one or more Bio reaction stages. This step is mainly consisting of three operations namely, production of biomass, metabolize biosynthesis and biotransformation. Finally, the material produced in the bioreactor must be further processed in the downstream section to convert it into more useful form. The downstream process is mainly consisting of physical separation operations which includes, solid liquid separation, adsorption, liquid-liquid extraction, distillation, drying etc.

The raw material can be of biological or non-biological origin. It is first converted to more suitable form for processing. This is done in upstream processing step which involves chemical hydrolysis, preparation of liquid medium, separation of particulate, air purification and many other preparatory operations.

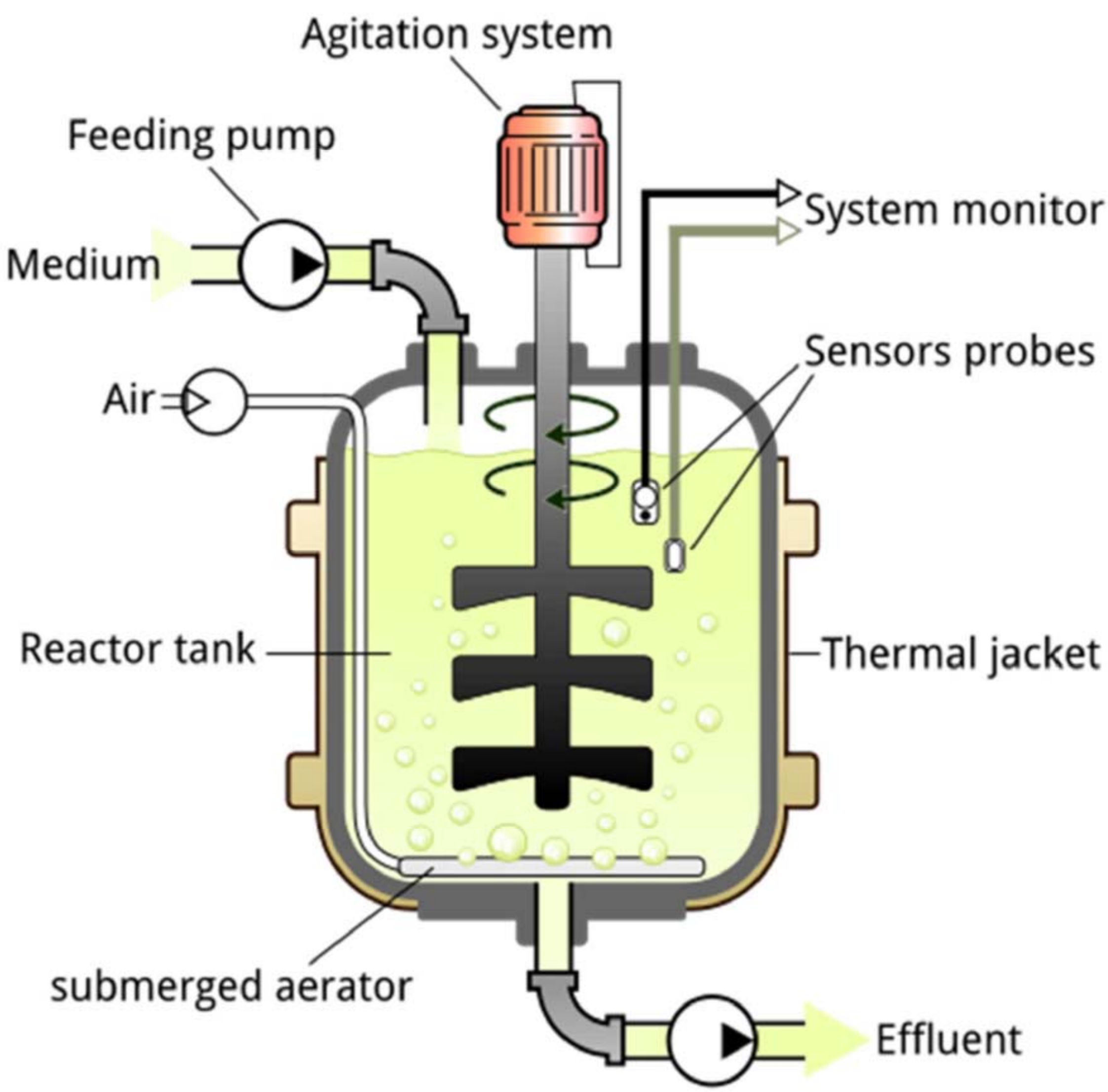
Bioreactors types:

1. Continuous Stirred Tank Bioreactors.
2. Bubble Column Bioreactor.
3. Airlift Bioreactors.
4. Fluidized Bed Bioreactors.
5. Packed Bed Bioreactors.
6. Photo-Bioreactors.

1. Continuous stirred tank bioreactors:

A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor, number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio. The aspect ratio of a stirred tank bioreactor is usually between 3-5, but for animal cell culture applications, the aspect ratio is less than 2. The diameter of the impeller is usually 1/3 rd of the vessel diameter, distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use.

General structure of a continuous stirred-tank type bioreactor

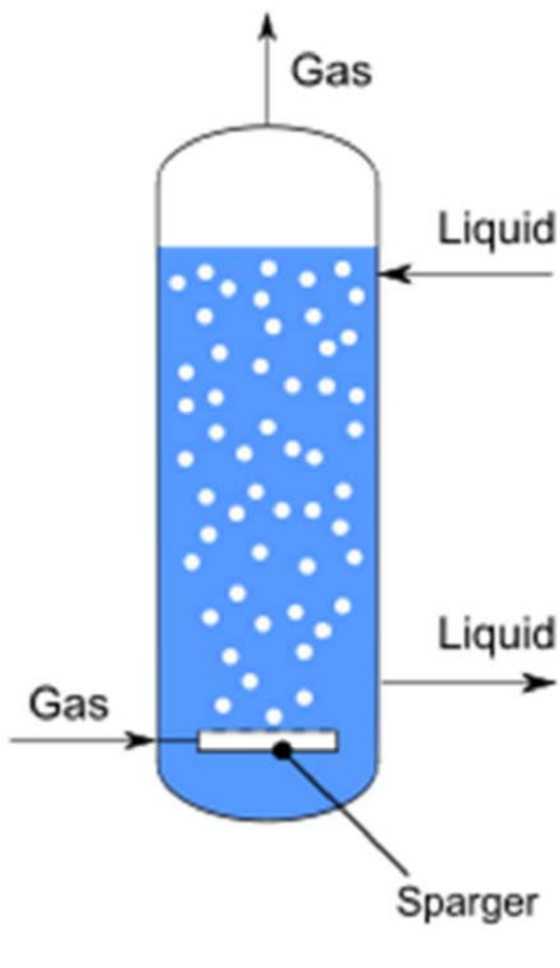


In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger. The sparger may be a ring with many holes or a tube with a single orifice, enabling better gas distribution system throughout the vessel with impellers (agitators). This enables the creation of a uniform and homogeneous environment throughout the bioreactor. There are many advantages of this type over others. These include the efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

2. Bubble column bioreactors:

In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro porous spargers. • The flow rate of the air/gas influences the performance factors —O2 transfer, mixing. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio).

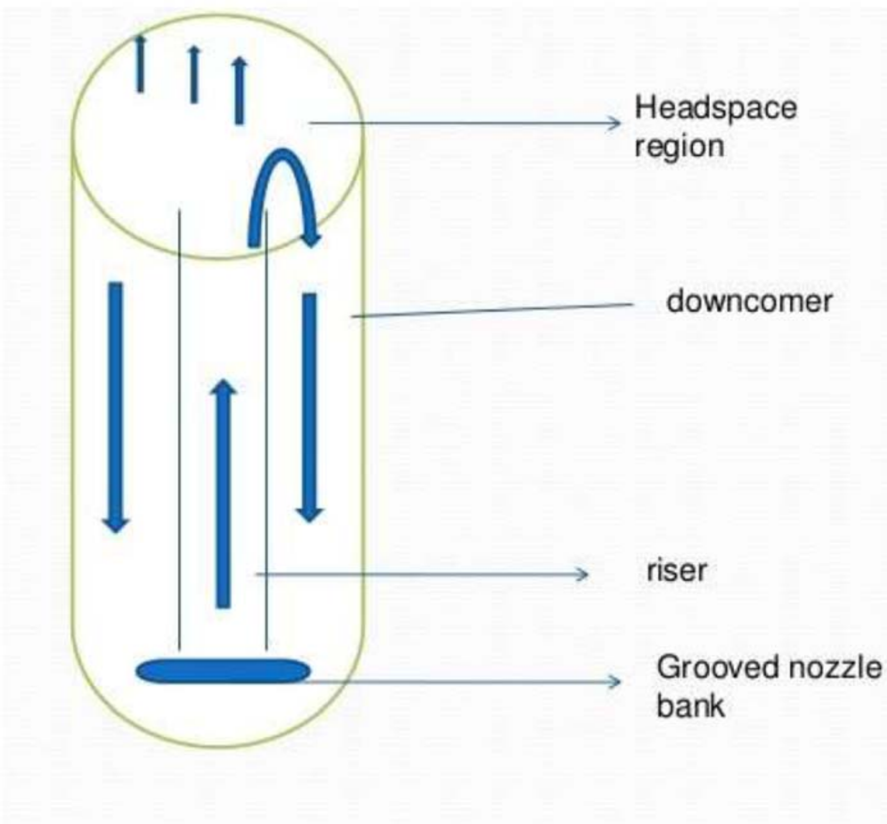
Diagram of bubble column bioreactor



3. Airlift bioreactors:

In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube, in one of the two zones referred to a riser, the air/gas is pumped. The other zone that receives no gas is the down comer. The diffusion flows up the riser zone while the down flow occurs in the down comer.

Diagram of Airlifted Bioreactor

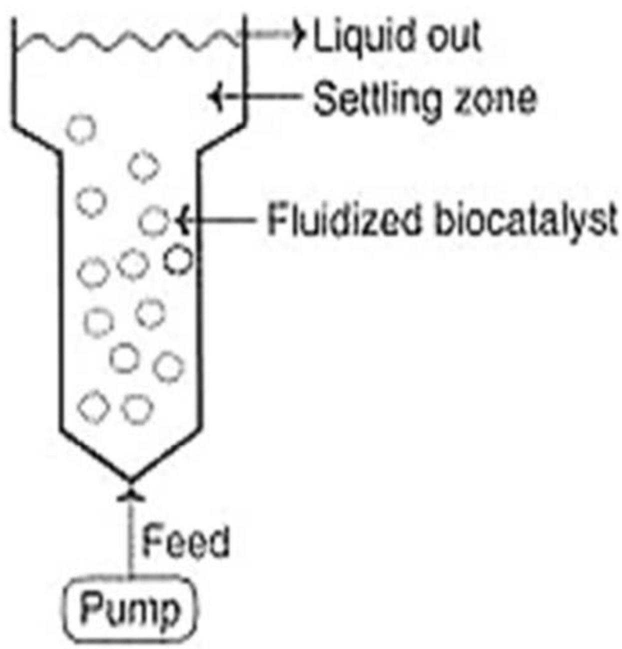


Airlift bioreactors are commonly employed for aerobic bioprocessing technology. They are preferred for methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

4. Fluidized bed bioreactors:

Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out. These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs.

Basic diagram of a fluidized bed reactor



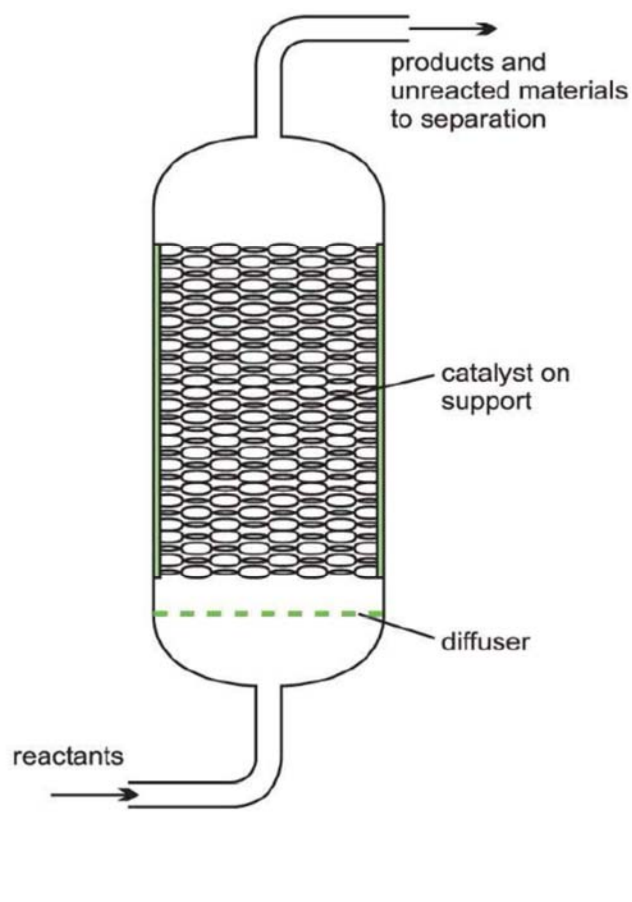
For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enable good efficiency of bioprocessing.

5. Packed bed bioreactors:

A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed. The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilized biocatalyst, obtained products in the packed bed bioreactor are released into the fluid and removed. • While the flow of the fluid can be upward or downward, down flow under gravity is preferred.

The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth. • Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. • However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions. • The packed bed bioreactors do not allow accumulation of the products to any significant extent.

Diagram of Packed bed bioreactors



6. Photo-bioreactors:

These are the bioreactors specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred. Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin. They are made up of glass or more commonly transparent plastic. The array of tubes or flat panels constitute light receiving systems (solar receivers). The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps. It is essential that the cells are in continuous circulation without forming sediments. tubes should also be cooled to prevent rise in temperature. Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25- 40°C. Microalgae and cyanobacteria are normally used. The organisms grow during day light while the products are produced during night.

A tubular photo-bioreactors with parallel run horizontal tubes.